EXHIBIT B

WOOD AND CELLULOSIC CHEMISTRY

second edition, revised and expanded

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Chemical Characterization of Wood and Its Components

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I. INTRODUCTION

Wood analysis comprises the determination of the wood components as well as the isolation, purification, and characterization of the wood constituents. Wood is chemically heterogeneous and its components can be divided into two groups: structural components of high molecular weight (cellulose, polyoses or hemicelluloses, and lignin), which are the major cell wall components; and nonstructural components of low molecular weight (extractives and inorganic compounds). The macromolecular components are not uniformly distributed in wood cells, and their concentration changes from one morphological region to another. Therefore, knowledge about the distribution of chemical components in the cell walls is of great importance to understanding the properties of wood. The chemical composition of wood varies from species to species. In general, hardwoods contain more hemicellulose than softwoods but less lignin. Figure 1 shows the typical composition of hardwoods and softwoods [1].

There are different types of wood analysis. One may consider only the main cell wall components, holocellulose (cellulose and polyoses), lignin, extractives, and ash; on the other hand, a very detailed analysis may include functional groups (e.g., acetyl groups), individual units of the different components (e.g., sugar pattern), different types and frequency of linkages (e.g., β-O-4 linkage in lignin), etc.

Usually, wood is analyzed by the separation of the different components, but there are serious difficulties in achieving selective isolations. The separation is never complete and leads to structural changes, predominantly in the lignin. An array of classical, wet chemical procedures and a growing number of instrumental methods are available for analysis of wood. The wet chemical methods permit the acquisition of data on the gross composition of wood, and they require the separation of wood into macroscopic wood components (e.g., lignin, holocellulose, etc.). This is the reason it is always necessary to report the isolation techniques used. On the other hand, the instrumental methods are conducive to higher specificity and convenience of wood analysis. Initially, chemical specificity was achieved in a macroscopic scale; for example, by chromatographic methods the separation and determination of the individual sugars can be obtained after hydrolysis of a wood sample. More recently, techniques such as ultraviolet microscopy, electron mi-

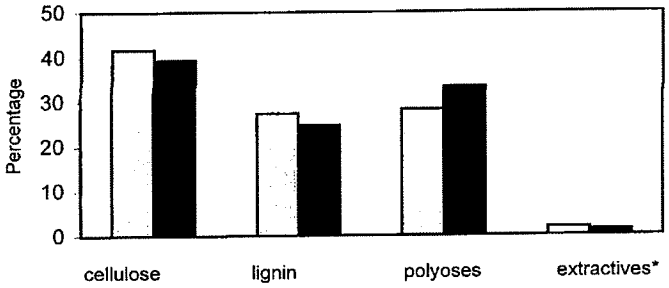

FIGURE 1 Typical composition of softwoods and hardwoods. ▩, Norway spruce (softwood); ■, common beech (hardwood). *CH_2Cl_2 followed by C_2H_5OH.

croscopy coupled with X-ray analysis, and infrared spectrometry have permitted descriptions of the distribution of chemical constituents in wood and fiber walls.

The methods of wood analysis are more or less standardized. Detailed descriptions of the analysis of wood are giving in the specialist literature [2–8]. The CPPA (Technical Section, Canadian Pulp and Paper Association, Montreal, PQ, Canada), TAPPI (Technical Association of the Pulp and Paper Industry, Atlanta, GA), ASTM (American Society of Testing and Materials, Philadelphia, PA) have issued new or revised test methods for the analysis of pulp and paper materials. There are excellent reviews covering analytical techniques for wood and its components [9–15].

The purpose of this chapter is to inform the reader about some of the methods available for the chemical analysis of wood. The analysis of the constituents of wood, according to the scheme shown in Fig. 2, will be described.

II. SUMMATIVE ANALYSIS

The objective of the summative analysis is to account for all of a sample. The summative analysis of wood is based on the isolation and identification of certain groups of wood components and therefore does not deal with the determination of chemical uniformity of substances. These groups are mainly cellulose, hemicelluloses, lignin, and extractives, and often other designations are added, e.g., holocellulose, α-cellulose, or sometimes terms originated from the method used (such as kraft lignin, Cross and Beavan cellulose).

In evaluating the results of the summative analysis, it is important to take into consideration the methods used. To obtain a complete mass balance of a wood sample, no constituents may be overlooked or repeated. Nothing should be determined by difference. Summative analysis will typically account for most of the wood components. Examples of summative analysis of wood composition have been reported elsewhere [16–21]. Summations may be taken in several ways, and some examples of different types of wood analyses are given in Table 1.

Values of 98–101 are generally acceptable, but frequently values deficient or in excess by about 10% are obtained [14,16,19,20]. Possible reasons for failure to achieve a complete mass balance have been reviewed by Browning [20]. Generally, the summative analysis is corrected via normalization, giving the same factor of error to all the components, independent of the amount in which the different components are present and in the analytical technique used. The possibility of undetected or unknown compounds in

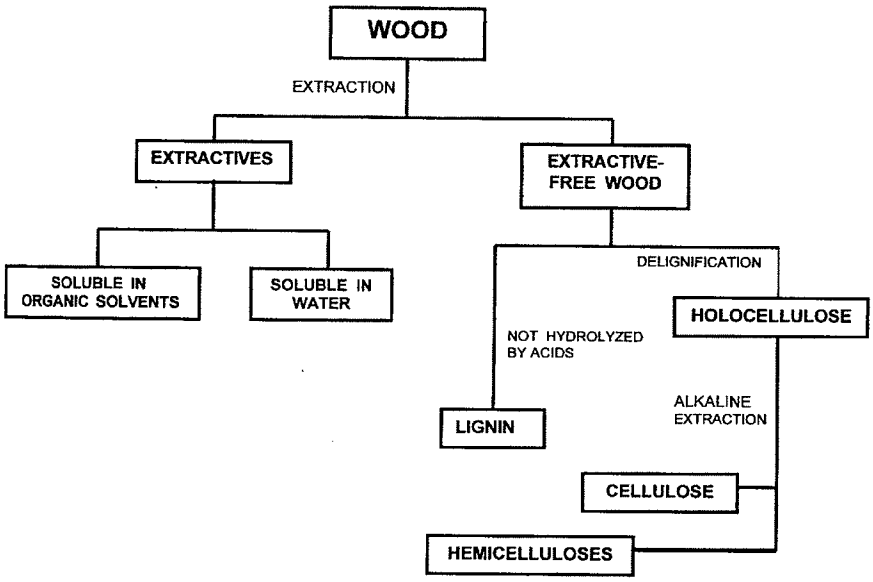

FIGURE 2 The separation of wood components.

sample wood is ignored. Kaar and Brink [21] developed a summative analysis scheme (bomb/HPLC summative analysis method) that provides a complete accounting of the starting material without normalization. This method utilizes sealed vessels to allow the retention of the volatiles during the high-temperature stage of hydrolysis, with the hydrolyzate analyzed by HPLC. There were no significant unidentified peaks in any of the HPLC chromatograms. The range of mass balance determinations for the group of 10 wood specimens on unextracted and extractive-free bases was 98.43–99.63% and 98.21–99.60%, respectively. The factors that could be responsible for the discrepancy from 100%

TABLE 1 Summative Analysis; Examples of Wood Analysis

A	B	C	D
Extractives	Extractives	Extractives	Extractives
Lignin	Lignin	Lignin	Lignin
Holocellulose	α-Cellulose	Glucan	Glucan
Ash	Hemicelluloses	Mannan	Mannan
	Acetyl groups	Galactan	Galactan
	Ash	Xylan	Xylan
		Arabinan	Arabinan
		Uronic anhydride	4-O-Me glucuronic ac.
		Ash	Glucuronic ac.
			Galacturonic ac.
			Acetyl groups
			Protein
			Ash

in the mass balance were extensively discussed. These factors include: the exact contribution of galacturonic acid in the wood; the methanol formed from the demethoxylation of polysaccharides and lignin; the loss of water in the lignin condensation/dehydration relations; the wood components included in the polysaccharides and/or lignin moieties that exist in such small quantities that they are not detected by the analytical methods utilized; and residual water that remains bonded in the wood after "oven drying" which is released in the hydrolytic stage and becomes part of the hydrolytic solution. Clearly, the contribution of any of these factors is small. The complex nature of wood seriously complicates the quantification of these contributions. The improvement of the methods and techniques of analysis could result in a mass balance close to 100%.

III. SAMPLING AND PREPARATION OF SAMPLES FOR ANALYSIS

The kind of sampling and sample preparation depends on many factors and on the aim of the analysis. Thus, the magnitude of the sampling needed for general characterization of a species is quite different than for the evaluation of trees in a specified stand. It is important to ensure that representative samples are collected which are free from outside contamination, and properly preserved. No analysis is better than the sample on which is based. However, for comparison of techniques and methods the only requirement is that the sample be uniform.

A standardized sampling procedure is given in TAPPI Standard T-257 cm-85 [2]. The procedure given is appropriate for wood in all forms, i.e., logs, chips, or sawdust. A probability sampling plan and an economic or engineered sampling plan are described. A detailed discussion of sampling and preparation of samples is given by Browning [22].

Wood for chemical analysis, after air drying, must be milled to achieve complete penetration by reagents and to ensure uniform reactions. Heating, preparation of very fine and dusty material, and regrinding coarse material must be avoided. Samples are screened and normally material passed through a 0.40-mm (40-mesh) sieve and retained on a 60-mesh sieve. The selected fraction should represent, if not the entire amount of material, at least 90–95% of the original sample. The extractive should be removed before any chemical analysis, except where the extraction process and subsequent washing could interfere with the analysis. A procedure for further preparation of wood for chemical analysis that has been sampled in accordance with TAPPI 257 is provided in TAPPI Test Method T264 om-88 [2]. Neutral solvents, ethanol and benzene, are employed to obtain extractive-free wood, removing material which is not part of the wood substance or which may interfere with subsequent analysis. Moisture determination is included. Related methods are ASTM D1105 (ANS), "Preparation of Extractive-Free Wood" [3]; and CPPA G.31P, "Preparation of Wood for Chemical Analysis" [4].

It is dangerous to include benzene in the solvent mixture to extract wood, due to its carcinogenic properties for which it has been long banned. Accordingly, any contact with the skin or inhalation of benzene vapor must be avoided. Extreme safety precautions must be taken in carrying out the above procedure. Gloves, good ventilation, and a chemical fume hood must be used.

A mixture of ethanol and toluene was found to remove the same materials from wood as ethanol–benzene [23]. However, the mixture of ethanol and toluene does not boil and reflux at a constant temperature and rate.

Wood samples collected for later analysis must remain moist and cold-stored. Samples should not be oven-dried to avoid changes in reactivity and lost of volatiles. After

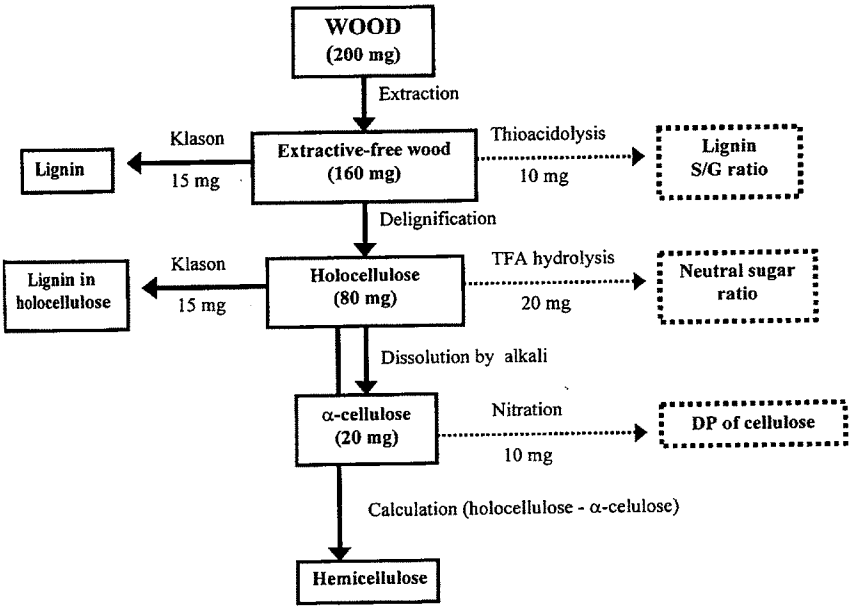

FIGURE 3 Experimental small-scale method. (From Ref. 24.)

air drying, most chemical properties of wood do not change under adequate storage conditions.

Recently, a small-scale method for the determination of wood components such as extractives, lignin, α-cellulose, and hemicellulose has been published [24]. This method is also used in the sample preparation for structural analysis of each component (Fig. 3).

IV. DETERMINATION OF WATER CONTENT

Water is a natural constituent of all parts of a living tree. In green wood, water is commonly about 50% of the total weight. When the tree dies or a log is processed into lumber, chips, etc., the wood loses some of its moisture to the surrounding atmosphere. However, some water will remain within the structure of the cell even after wood has been manufactured into lumber, particle, veneer, or fiber product. The amount of residual water depends on the extent of drying and the environmental conditions. The wood–water system is very important in many fields of wood technology. The physical and mechanical properties, resistance to biological deterioration, and dimensional stability of the products are affected by the amount of water present.

Chemical analysis of wood is almost always performed on air-dried samples, but results are reported on a moisture-free basis. A moisture determination must therefore be run for almost every sample submitted for analysis.

The amount of water in wood is expressed in two ways:

technique simultaneous multielement analysis can be achieved by using solid samples (wood, sawdust, ash).

The specific locations of elements in intact samples may be determined by an X-ray analysis attaching to a scanning electron microscope [638]. By imaging-microprobe secondary-ion mass spectrometry (SIMS), the spatial distribution of the elements may be determined. The spatial distribution of trace elements in jack pine, *Pinus basksiana* (lamb), by SIMS was determined [639]. Trace elements were found to be concentrated in specific morphological features, namely, the torus, middle lamella, cell corners, and ray parenchyma wall. The samples of jack pine were examined for Ca, Mn, Cu, and Zn by NAA and/or ICP-AES and for Fe, K, Al, Cl, Mg, Sr, and Cr by ICP-AES. Although differences in environmental conditions during the growth of a tree can result in large variations in the concentration of trace elements, the values obtained from this study are in agreement, within experimental error, with values obtained from the bulk inorganic content of jack pine [640,641].

Saka and Göring [642] studied the distribution of inorganic constituents of black spruce (*Picea mariana* Mill.) by means of transmission scanning microscopy coupled with energy disperse X-ray analysis (TEM-EDXA) and detected 14 elements (Na, Mg, Al, Si, S, Cl, K, Ca, Cr, Fe, Ni, Cu, Zn, and Pb). Almost all of these elements detected were found to be concentrated in the torus and half-bordered pit membrane regions. In the secondary walls of tracheids, ray tracheids, and ray parenchyma cells, only S, Cl, K, and Ca were detected. Saka and Mimori [643], by scanning microscopy coupled with EDXA (SEM-EDXA), determined the distribution of inorganic constituents in Japanese birch wood (*Betulo platyphylla* Sukatchev var. *japonica* Hara). Six morphological regions of the wood fibers, vessels, and ray parenchyma cells were investigated, and up to 11 different elements (Na, Mg, Al, Si, P, S, Cl, K, Ca, Fe, and Zn) were detected. The secondary walls of wood fibers, vessels, ray tracheids, and ray parenchyma cells usually contain only detectable concentrations of S, Cl, and Ca. In contrast, almost all of these elements detected were found to be localized and concentrated in the amorphous layers of ray parenchyma cells and pit membranes between vessels and ray parenchyma cells. The content of inorganic constituents determined by SEM-EDXA is in good agreement with the results obtained from ash residues of wood by bulk analysis.

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4. *CPPA Standards*, Canadian Pulp and Paper Association, Montreal, Quebec, Canada.
5. *Appita Standards*, Australian Pulp and Paper Industry Technical Association.
6. *ISO Standards*, International Organization for Standardization.
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